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Transmitted herewith for filing under 37 CFR §1.53(c) is the PROVISIONAL APPLICATION for patent of

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TITLE OF THE INVENTION (280 characters max)

ONCOLYTIC ADENOVIRAL VECTORS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

- ☒ Specification (Including Any Claims and Abstract) - 44 pages
- ☐ Drawings - sheets
- ☒ Other (specify): Sequence Listing (paper copy [3 pages]); diskette containing the Sequence Listing and Verification; Inventor Information Sheet; Return Postcard

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IN RE APPLICATION OF
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SUBMISSION OF SEQUENCE LISTING
INCLUDING STATEMENT OF VERIFICATION

Sir:

Applicant hereby provides a Computer Readable Form of the Sequence Listing as well as the Paper Copy thereof. The undersigned states that the Paper Copy and the Computer Readable Form, submitted in accordance with 37 CFR §1.821(c) and (e), respectively, are the same.

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ONCOLYTIC ADENOVIRAL VECTORS

FIELD OF THE INVENTION

The present invention generally relates to substances and methods useful for the treatment of neoplastic disease. More specifically, it relates to cancer selective promoters and their use in oncolytic adenoviral vectors. The oncolytic adenoviral vectors are useful in methods of gene therapy. The promoters and the oncolytic adenoviral vectors are also useful in methods of screening for compounds that modulate the expression of cancer selective genes.

BACKGROUND OF THE INVENTION

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated herein by reference.

Adenoviruses that replicate selectively in tumor cells are being developed as anticancer agents ("oncolytic adenoviruses"). Such oncolytic adenoviruses amplify the input virus dose due to viral replication in the tumor, leading to spread of the virus throughout the tumor mass. In situ replication of adenoviruses leads to cell lysis. This in situ replication may allow relatively low, non-toxic doses to be highly effective in the selective elimination of tumor cells.

One approach to achieving selectivity is to introduce loss-of-function mutations in viral genes that are essential for growth in non-target cells but not in tumor cells. This strategy is exemplified by the use of Add*l*1520, which has a deletion in the E1b-55KD gene. In normal cells, the adenoviral E1b-55KD protein is needed to bind to p53 to prevent apoptosis. In p53-deficient tumor cells, E1b-55K binding to p53 is unnecessary. Thus, deletion of E1b-55KD should theoretically restrict vector replication to p53-deficient tumor cells.

Another approach is to use tumor-selective to control the expression of early viral genes required for replication. (See, e.g., WO 96/17053, WO 99/25860, WO 02/067861, WO 02/068627, and U.S. Patent Nos. 5,698,443, 5,871,726, 5,998,205, and 6,432,700, all of which are incorporated herein by reference). Thus, in this approach the adenoviruses will selectively replicate and lyse tumor cells if the gene that is essential for replication is under the control of a promoter or other transcriptional regulatory element that is tumor-selective.

The *TMPRSS2* gene encodes a predicted protein of 492 amino acids, with five distinct domains (Paoloni-Giacobino *et al.*, *Genomics*, 1997, 44:309-320). On the basis of homology to other proteins, these regions comprise a serine protease domain of the S1 family; a scavenger receptor cysteine-rich domain, possibly involved in binding to cell surface molecules; a class A low-density lipoprotein domain; a transmembrane domain; and a cytoplasmic domain (Paoloni-Giacobino *et al.*, *Genomics*, 1997, 44:309-320.) Analysis of the predicted TMPRSS2 protein indicates that TMPRSS2 is a type II integral membrane protein that is most similar to the mammalian hepsins, a class of proteins that are important for cell growth and maintenance of normal cell morphology (Kurachi *et al.*, *Methods Enzymology*, 1994, 244:100-114). Hepsin has been shown to be overexpressed in ovarian carcinomas and is suggested to be a candidate mediator of the invasive process and growth capacity of ovarian tumor cells (Tanimoto *et al.*, *Cancer Res*, 1997, 57:2884-2887).

The features of the protease domain of TMPRSS2 are compatible with the S1 family of serine proteases with chymotrypsin as the prototype member (Rawlings *et al.*, *Methods Enzymology*, 1994, 244:19-61). The Asp^{435} residue in the substrate-binding site indicates that *TMPRSS2* will have trypsin-like proteolytic activity, with cleavage after Lys or Arg residues. Thus, TMPRSS2 could be a natural activator of the precursor forms of PSA and hK2. The zymogen form of TMPRSS2 is also predicted to be proteolyzed by trypsin-like enzymes that cleave the Arg-Ile bond of the NH_2 terminus of the protease domain. Thus, TMPRSS2 could be autocatalytic or could be activated by other trypsin-like proteases in the prostate, such as hK2 or prostase/PRSS17. The characterization of TMPRSS2 as a serine protease expressed in prostate epithelium adds another member to a group of proteases that may participate in a cascade mechanism of enzymatic reactions in seminal fluid that is analogous to the fibrinolytic and blood coagulation system (Davie *et al.*, *Biochemistry*, 1991 **30**:10363-10370; Takayama *et al.*, *J Biol Chem*, 1997, 272:21582-21588).

SUMMARY OF THE INVENTION

The present invention exploits the discovery, that the *TMPRSS2* promoter has tumor selective properties, which is valuable not only for the development of therapeutic molecules but also for research in cancer biology, carcinogenesis and functional genomics. Thus, the present invention provides novel and improved oncolytic adenoviral vectors and their uses in methods of gene therapy. The present invention includes oncolytic adenoviral vectors comprising a cancer

selective regulatory region operatively linked to an adenoviral gene essential for adenoviral replication. Adenoviral genes essential for replication include, but are not limited to, E1a, E1b, E2a, E2b and E4. In one embodiment, the oncolytic adenoviral vector has a cancer selective regulatory region operatively linked to the E1a gene. With respect to the present invention, the cancer selective regulatory region is a *TMPRSS2* promoter. In another embodiment, the oncolytic adenoviral vector has a *TMPRSS2* promoter operatively linked to the E1a gene and a second cancer selective regulatory region operatively linked to the E4 gene. In another embodiment, the oncolytic adenoviral vector has a *TMPRSS2* promoter operatively linked to the E4 gene and a second cancer selective regulatory region operatively linked to the E1a gene. In yet another embodiment, the oncolytic adenoviral vector carries at least one therapeutic transgene, *e.g.* a polynucleotide encoding a cytokine such as GM-CSF that can stimulate a systemic immune response against tumor cells.

Accordingly, in one aspect, the present invention provides an isolated *TMPRSS2* promoter comprising a nucleotide sequence selected from the group consisting of: (a) the sequence shown in SEQ ID NO:1; (b) a fragment of the sequence shown in SEQ ID NO:1, wherein the fragment has tumor selective promoter activity; (c) a nucleotide sequence having at least 90% identity over its entire length to the sequence shown in SEQ ID NO:1, wherein the nucleotide sequence has tumor selective promoter activity; and (d) a nucleotide sequence having a full-length complement that hybridizes under stringent conditions to the sequence shown in SEQ ID NO:1, wherein the nucleotide sequence has tumor selective promoter activity. In another embodiment, the *TMPRSS2* promoter consists essentially of SEQ ID NO:1. Preferably, a a *TMPRSS2* promoter according to the invention is prostate tumor selective.

The present invention also provides a chimeric gene comprising a *TMPRSS2* promoter according to the invention operatively linked to the coding sequence of a gene of interest.

The present invention further provides a vector comprising a chimeric gene according to the invention. Preferably, the vector is a viral vector. More preferably, the vector is an adenoviral vector.

The present invention still further provides an isolated cell such as a packaging cell tranduced with a vector according to the invention.

In another aspect, the present invention provides a recombinant viral vector comprising an adenoviral nucleic acid backbone comprising: a left ITR, an adenoviral packaging signal, a *TMPRSS2* promoter operatively linked to the coding sequence of a gene essential for replication of the recombinant viral vector, and a right ITR. In one embodiment, the adenoviral nucleic acid

backbone comprises in sequential order: a left ITR, a *TMPRSS2* promoter of the invention operatively linked to the coding sequence of a gene essential for replication of the recombinant viral vector, an adenoviral packaging signal, and a right ITR. In another embodiment, the adenoviral nucleic acid backbone comprises in sequential order: a left ITR, an adenoviral packaging signal, a *TMPRSS2* promoter of the invention operatively linked to the coding sequence of a gene essential for replication of the recombinant viral vector, and a right ITR.

In one embodiment of a recombinant viral vector of the invention, the *TMPRSS2* promoter is a human *TMPRSS2* promoter. In another embodiment of a recombinant viral vector of the invention, the *TMPRSS2* promoter comprises a nucleotide sequence selected from the group consisting of: (a) the sequence shown in SEQ ID NO:1; (b) a fragment of the sequence shown in SEQ ID NO:1, wherein the fragment has tumor selective promoter activity; (c) a nucleotide sequence having at least 90% identity over its entire length to the sequence shown in SEQ ID NO:1, wherein the nucleotide sequence has tumor selective promoter activity; and (d) a nucleotide sequence having a full-length complement that hybridizes under stringent conditions to the sequence shown in SEQ ID NO:1, wherein the nucleotide sequence has tumor selective promoter activity. In another embodiment of a recombinant viral vector of the invention, the *TMPRSS2* promoter consists essentially of SEQ ID NO:1.

In a preferred embodiment of a recombinant viral vector of the invention, the coding sequence of a gene essential for replication is selected from the group consisting of E1a, E1b, E2a, E2b and E4 coding sequences. In one embodiment, the *TMPRSS2* promoter is operatively linked to one of either the E1a coding sequence or the E4 coding sequence. In another embodiment, the vector further comprises a tissue-selective promoter, *e.g.* an *hTERT* promoter, an E2F-1 promoter, or an osteocalcin promoter, operatively linked to the other of the E1a coding sequence or the E4 coding sequence. For example, the tissue-selective promoter operatively may be an *hTERT* promoter comprising SEQ ID NO:2 or SEQ ID NO:3. Preferably, the *TMPRSS2* promoter is operatively linked to the E1a coding sequence and the tissue-selective promoter is operatively linked to the E4 coding sequence.

In another embodiment of a recombinant viral vector of the invention, the adenoviral nucleic acid backbone is derived from adenovirus serotype 5 (Ad5) or serotype 35 (Ad35).

In another embodiment of a recombinant viral vector of the invention, the nucleic acid backbone further comprises a termination signal sequence upstream of the *TMPRSS2* promoter operatively linked to the coding sequence of a gene essential for replication of the recombinant viral vector. In one embodiment, the termination signal sequence is the SV40 early

polyadenylation signal sequence. In another embodiment, the vector further comprises a deletion upstream of the termination signal sequence. For example, the vector may comprise a deletion between nucleotides corresponding to nucleotides 103 and 551 of the adenoviral type 5 backbone.

In another embodiment of a recombinant viral vector of the invention, the E1b gene of the adenoviral nucleic acid backbone has been mutated or deleted. In this embodiment, mutation or deletion of the E1b gene preferably results in the loss of the active 19kD protein expressed by the wild-type E1b gene.

In another embodiment of a recombinant viral vector of the invention, the E3 region of the adenoviral nucleic acid backbone has been mutated or deleted.

In another embodiment of a recombinant viral vector of the invention, the recombinant viral vector further comprises a heterologous coding sequence. In one embodiment, the heterologous coding sequence is inserted in the E3 region of the adenoviral nucleic acid backbone. For example, the heterologous coding sequence may be inserted in place of the 19kD or 14.7 kD E3 gene. In one embodiment, the heterologous coding sequence encodes an immunostimulatory protein. In another embodiment, the immunostimulatory protein is a cytokine such as GM-CSF. In yet another embodiment, the heterologous coding sequence encodes an anti-angiogenic protein. In still another embodiment, the heterologous coding sequence is a suicide gene.

In a preferred embodiment, a recombinant viral vector of the invention selectively replicates in tumor cells. In a more preferred embodiment, a recombinant viral vector of the invention selectively replicates in prostate tumor cells. In an exemplary embodiment, tumor-selectivity is at least about 3-fold as measured by E1a RNA levels in infected tumor vs. infected non-tumor cells.

In yet another aspect, the present invention provides a recombinant adenovirus particle comprising a recombinant viral vector according to the invention. In one embodiment, a capsid protein of the adenovirus particle comprises a targeting ligand. In another embodiment, the capsid protein is a fiber protein. In yet another embodiment, the targeting ligand is in the HI loop of the fiber protein.

In still another aspect, the present invention provides a method of selectively killing a neoplastic cell, comprising contacting an effective number of recombinant adenovirus particles according to the invention with the cell under conditions where the recombinant adenovirus particles can transduce the cell. In one embodiment, the neoplastic cell is a prostate tumor cell.

In another aspect, the present invention provides a pharmaceutical composition comprising the recombinant adenovirus particle according to the invention and a pharmaceutically acceptable carrier.

In another aspect, the present invention provides a method of treating a host organism having a neoplastic condition, comprising administering a therapeutically effective amount of the pharmaceutical composition according to the invention to the host organism. Preferably, the host organism is a human patient. In one embodiment, the neoplastic condition is prostate cancer. According to one embodiment, administering a therapeutically effective amount of the pharmaceutical composition to the host organism comprises an intratumoral injection of a therapeutically effective dosage of the composition. According to another embodiment, administering a therapeutically effective amount of the pharmaceutical composition to the host organism comprises systemic administration of a therapeutically effective dosage of the composition.

The present invention also provides methods for screening compounds that are useful for modulating the expression of TMPRSS2 in cancer tissue. In one embodiment, the method of screening compounds includes comparing the level of TMPRSS2 expression in the absence of the compound to the level of expression in the presence of the drug candidate, wherein the concentration of the compound can vary when present, and wherein the comparison can occur after addition or removal of the compound. The method may utilize eukaryotic or prokaryotic host cells that are stably transformed with recombinant polynucleotides comprising a regulatory region of the *TMPRSS2* gene operatively linked to a nucleic acid sequence encoding a product that can be detected. A candidate compound is added to the host cells and the expression of the detectable product is compared to a control.

The present invention further provides a method that utilizes host cells transduced with adenoviral vectors comprising a *TMPRSS2* promoter of the invention operatively linked to an essential adenoviral gene, *e.g.* E1a, for screening compounds useful for modulating the expression of TMPRSS2 in cancer tissue. According to this method, a candidate compound is added to the host cells and expression of the essential adenoviral gene or viral replication is detected and compared to a control.

DESCRIPTION OF THE SEQUENCES IN THE SEQUENCE LISTING

The Sequence Listing associated with the instant disclosure is hereby incorporated by reference into the instant disclosure. The following is a description of the sequences contained in the Sequence Listing:

SEQ ID NO:1 is a 1992 bp fragment of the human *TMPRSS2* promoter.

SEQ ID NO:2 is a 397 bp fragment of the human *TERT* promoter.

SEQ ID NO:3 is a 245 bp fragment of the human *TERT* promoter.

SEQ ID NO:4 through SEQ ID NO:7 are PCR primers.

DETAILED DESCRIPTION OF THE INVENTION

In describing the present invention, the following terms are employed and are intended to be defined as indicated below.

The terms "virus," "viral particle," "vector particle," "viral vector particle," and "virion" are used interchangeably and are to be understood broadly as meaning infectious viral particles that are formed when, *e.g.*, a viral vector of the invention is transduced into an appropriate cell or cell line for the generation of infectious particles. Viral particles according to the invention may be utilized for the purpose of transferring DNA into cells either *in vitro* or *in vivo*. For purposes of the present invention, these terms preferably refer to adenoviruses, including recombinant adenoviruses formed when an adenoviral vector of the invention is encapsulated in an adenovirus capsid.

As used herein, the terms "adenovirus" and "adenoviral particle" are used to include any and all viruses that may be categorized as an adenovirus, including any adenovirus that infects a human or an animal, including all groups, subgroups, and serotypes. Thus, as used herein, "adenovirus" and "adenovirus particle" refer to the virus itself or derivatives thereof and cover all serotypes and subtypes and both naturally occurring and recombinant forms, except where indicated otherwise. Preferably, such adenoviruses are ones that infect human cells. Such adenoviruses may be wildtype or may be modified in various ways known in the art or as disclosed herein. Such modifications include modifications to the adenovirus genome that is packaged in the particle in order to make an infectious virus. Such modifications include deletions known in the art, such as deletions in one or more of the E1a, E1b, E2a, E2b, E3, or E4 coding regions. Such modifications also include deletions of all of the coding regions of the

adenoviral genome. Such adenoviruses are known as "gutless" adenoviruses. The terms also include replication-conditional adenoviruses; that is, viruses that preferentially replicate in certain types of cells or tissues but to a lesser degree or not at all in other types. In a preferred embodiment of the invention, the adenoviral particles selectively replicate in abnormally proliferating tissue, such as solid tumors and other neoplasms. These include the viruses disclosed in U.S. Patent Nos. 5,677,178, 5,698,443, 5,871,726, 5,801,029, 5,998,205, and 6,432,700, the disclosures of which are incorporated herein by reference in their entirety. Such viruses are sometimes referred to as "cytolytic" or "cytopathic" viruses (or vectors), and, if they have such an effect on neoplastic cells, are referred to as "oncolytic" viruses (or vectors).

The terms "vector," "polynucleotide vector," "polynucleotide vector construct," "nucleic acid vector construct," and "vector construct" are used interchangeably herein to mean any nucleic acid construct for gene transfer, as understood by one skilled in the art.

As used herein, the term "viral vector" is used according to its art-recognized meaning. It refers to a nucleic acid vector construct that includes at least one element of viral origin and may be packaged into a viral vector particle. The viral vector particles may be utilized for the purpose of transferring DNA, RNA or other nucleic acids into cells either *in vitro* or *in vivo*. Viral vectors include, but are not limited to, retroviral vectors, vaccinia vectors, lentiviral vectors, herpes virus vectors (*e.g.*, HSV), baculoviral vectors, cytomegalovirus (CMV) vectors, papillomavirus vectors, simian virus (SV40) vectors, Sindbis vectors, semliki forest virus vectors, phage vectors, adenoviral vectors, and adeno-associated viral (AAV) vectors. Suitable viral vectors are described in U.S. Patent Nos. 6,057,155, 5,543,328 and 5,756,086. For purposes of the present invention, the viral vector is preferably an adenoviral vector.

The terms "adenovirus vector" and "adenoviral vector" are used interchangeably and are well understood in the art to mean a polynucleotide comprising all or a portion of an adenovirus genome. An adenoviral vector of this invention may be in any of several forms, including, but not limited to, naked DNA, DNA encapsulated in an adenovirus capsid, DNA packaged in another viral or viral-like form (such as herpes simplex, and AAV), DNA encapsulated in liposomes, DNA complexed with polylysine, complexed with synthetic polycationic molecules, conjugated with transferrin, complexed with compounds such as PEG to immunologically "mask" the molecule and/or increase half-life, or conjugated to a non-viral protein.

In the context of adenoviral vectors, the term "5'" is used interchangeably with "upstream" and means in the direction of the left inverted terminal repeat (ITR). In the context

of adenoviral vectors, the term "3'" is used interchangeably with "downstream" and means in the direction of the right ITR.

As used herein, the terms "cancer," "cancer cells," "neoplastic cells," "neoplasia," "tumor," and "tumor cells" (used interchangeably) refer to cells that exhibit relatively autonomous growth, so that they exhibit an aberrant growth phenotype characterized by a significant loss of control of cell proliferation. Neoplastic cells can be malignant or benign.

The terms "chimeric gene" and "chimeric construct" refer to a recombinant DNA sequence in which a promoter or regulatory DNA sequence is operatively linked to a DNA sequence that codes for an mRNA or that is expressed as a protein, such that the promoter or regulatory DNA sequence is able to regulate transcription or expression of the associated DNA sequence. The regulator DNA sequence of the chimeric gene or chimeric construct is not normally operatively linked to the associated DNA sequence as found in nature.

The terms "coding sequence" and "coding region" refer to a nucleic acid sequence that is transcribed into RNA such as mRNA, rRNA, tRNA, snRNA, sense RNA or antisense RNA. Preferably the RNA is then translated in a cell to produce a protein.

The terms "complement" and "complementary" refer to two nucleotide sequences that comprise antiparallel nucleotide sequences capable of pairing with one another upon formation of hydrogen bonds between the complementary base residues in the antiparallel nucleotide sequences.

The term "consists essentially of" as used herein with reference to a particular nucleotide sequence means that the particular sequence may have up to 20 additional residues on either the 5' or 3' end or both, wherein the additional residues do not materially affect the basic and novel characteristics of the recited sequence.

The term "enhancer" within the meaning of the invention may be any genetic element, *e.g.*, a nucleotide sequence, that increases transcription of a coding sequence operatively linked to a promoter to an extent greater than the transcription activation effected by the promoter itself when operatively linked to the coding sequence, *i.e.* it increases transcription from the promoter.

The term "expression" refers to the transcription and/or translation of an endogenous gene or a transgene in a cell. In the case of an antisense construct, expression may refer to the transcription of the antisense DNA only.

The term "gene" refers to a defined region that is located within a genome and that, in addition to the aforementioned coding sequence, comprises other, primarily regulatory, nucleic acid sequences responsible for the control of expression, *i.e.*, transcription and translation of the

coding portion. A gene may also comprise other 5' and 3' untranslated sequences and termination sequences. Depending on the source of the gene, further elements that may be present are, for example, introns.

The term "gene essential for replication" refers to a nucleic acid sequence whose transcription is required for a viral vector to replicate in a target cell. For example, in an adenoviral vector of the invention, a gene essential for replication may be selected from the group consisting of the E1a, E1b, E2a, E2b, and E4 genes. Most preferably, the gene essential for replication is selected from the group consisting of the E1a, E1b, and E4 genes. Particularly preferred is the adenoviral E1a gene as the gene essential for replication.

The terms "heterologous" and "exogenous" as used herein with reference to nucleic acid molecules such as promoters and gene coding sequences, refer to sequences that originate from a source foreign to a particular virus or host cell or, if from the same source, are modified from their original form. Thus, a heterologous gene in a virus or cell includes a gene that is endogenous to the particular virus or cell but has been modified through, for example, codon optimization. The terms also includes non-naturally occurring multiple copies of a naturally occurring nucleic acid sequence. Thus, the terms refer to a nucleic acid segment that is foreign or heterologous to the virus or cell, or homologous to the virus or cell but in a position within the host viral or cellular genome in which it is not ordinarily found.

The term "homologous" as used herein with reference to a nucleic acid molecule refers to a nucleic acid sequence naturally associated with a host virus or cell.

The terms "identical" or percent "identity" in the context of two or more nucleic acid or protein sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described herein, *e.g.* the Smith-Waterman algorithm, or by visual inspection.

In the context of the present invention, the term "isolated" refers to a nucleic acid molecule, polypeptide, virus, or cell that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated nucleic acid molecule or polypeptide may exist in a purified form or may exist in a non-native environment such as, for example, a recombinant host cell. An isolated virus or cell may exist in a purified form, such as in a cell culture, or may exist in a non-native environment such as, for example, a recombinant or xenogeneic organism.

The term "native" refers to a gene that is present in the genome of wildtype virus or cell.

The term "naturally occurring" or "wildtype" is used to describe an object that can be found in nature as distinct from being artificially produced by man. For example, a protein or nucleotide sequence present in an organism (including a virus), which can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory, is naturally occurring.

The term "nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof ("polynucleotides") in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid molecule/polynucleotide also implicitly encompasses conservatively modified variants thereof (*e.g.* degenerate codon substitutions) and complementary sequences and as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, Nucleic Acid Res. 19: 5081 (1991); Ohtsuka *et al.*, J. Biol. Chem. 260: 2605-2608 (1985); Rossolini *et al.*, Mol. Cell. Probes 8: 91-98 (1994)). In the context of the present invention, the nucleic acid molecule/polynucleotide is preferably a segment of DNA. Nucleotides are indicated by their bases by the following standard abbreviations: adenine (A), cytosine (C), thymine (T), and guanine (G).

A nucleic acid sequence is "operatively linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, a promoter or regulatory DNA sequence is said to be "operatively linked" to a DNA sequence that codes for an RNA or a protein if the two sequences are operatively linked, or situated such that the promoter or regulatory DNA sequence affects the expression level of the coding or structural DNA sequence. Operatively linked DNA sequences are typically, but not necessarily, contiguous.

The term "ORF" means Open Reading Frame.

As used herein, a "packaging cell" is a cell that is able to package adenoviral genomes or modified genomes to produce viral particles. It can provide a missing gene product or its equivalent. Thus, packaging cells can provide complementing functions for the genes deleted in an adenoviral genome and are able to package the adenoviral genomes into the adenovirus particle. The production of such particles require that the genome be replicated and that those proteins necessary for assembling an infectious virus are produced. The particles also can

require certain proteins necessary for the maturation of the viral particle. Such proteins can be provided by the vector or by the packaging cell.

The term "promoter" refers to an untranslated DNA sequence usually located upstream of the coding region that contains the binding site for RNA polymerase II and initiates transcription of the DNA. The promoter region may also include other elements that act as regulators of gene expression. The term "minimal promoter" refers to a promoter element, particularly a TATA element, that is inactive or has greatly reduced promoter activity in the absence of upstream activation elements.

The term "recombinant" as used herein with reference to nucleic acid molecules refers to a combination of nucleic acid molecules that are joined together using recombinant DNA technology into a progeny nucleic acid molecule. As used herein with reference to viruses, cells, and organisms, the terms "recombinant," "transformed," and "transgenic" refer to a host virus, cell, or organism into which a heterologous nucleic acid molecule has been introduced. The nucleic acid molecule can be stably integrated into the genome of the host or the nucleic acid molecule can also be present as an extrachromosomal molecule. Such an extrachromosomal molecule can be auto-replicating. Recombinant viruses, cells, and organisms are understood to encompass not only the end product of a transformation process, but also recombinant progeny thereof. A "non-transformed," "non-transgenic," or "non-recombinant" host refers to a wildtype virus, cell, or organism that does not contain the heterologous nucleic acid molecule.

"Regulatory elements" are sequences involved in controlling the expression of a nucleotide sequence. Regulatory elements include promoters, enhancers, and termination signals. They also typically encompass sequences required for proper translation of the nucleotide sequence.

A "selectable marker gene" is a gene whose expression in a cell gives the cell a selective advantage. The selective advantage possessed by the cells transformed with the selectable marker gene may be due to their ability to grow in the presence of a negative selective agent, such as an antibiotic, compared to the growth of non-transformed cells. The selective advantage possessed by the transformed cells, compared to non-transformed cells, may also be due to their enhanced or novel capacity to utilize an added compound as a nutrient, growth factor or energy source.

A "termination signal sequence" within the meaning of the invention may be any genetic element that causes RNA polymerase to terminate transcription, such as for example a polyadenylation signal sequence. A polyadenylation signal sequence is a recognition region

necessary for endonuclease cleavage of an RNA transcript that is followed by the polyadenylation consensus sequence AATAAA. A polyadenylation signal sequence provides a "polyA site", *i.e.* a site on a RNA transcript to which adenine residues will be added by post-transcriptional polyadenylation. Polyadenylation signal sequences are useful insulating sequences for transcription units within eukaryotic cells and eukaryotic viruses. Generally, the polyadenylation signal sequence includes a core poly(A) signal that consists of two recognition elements flanking a cleavage-polyadenylation site (*e.g.*, Figure 1 of WO 02/067861 and WO 02/068627). Typically, an almost invariant AAUAAA hexamer lies 20 to 50 nucleotides upstream of a more variable element rich in U or GU residues. Cleavage between these two elements is usually on the 3' side of an A residue and, *in vitro*, is mediated by a large, multicomponent protein complex. The choice of a suitable polyadenylation signal sequence will consider the strength of the polyadenylation signal sequence, as completion of polyadenylation process correlates with poly(A) site strength (Chao *et al.*, *Molecular and Cellular Biology*, 1999, 19:5588-5600). For example, the strong SV40 late poly(A) site is committed to cleavage more rapidly than the weaker SV40 early poly(A) site. The person skilled in the art will consider to choose a stronger polyadenylation signal sequence if a more substantive reduction of nonspecific transcription is required in a particular vector construct. In principle, any polyadenylation signal sequence may be useful for the purposes of the present invention. However, in preferred embodiments of this invention the termination signal sequence is either the SV40 late polyadenylation signal sequence or the SV40 early polyadenylation signal sequence. Preferably, the termination signal sequence is isolated from its genetic source and inserted into the viral vector at a suitable position upstream of a *TMPRSS2* promoter.

The term "*TMPRSS2* promoter" refers to the native *TMPRSS2* promoter and functional fragments, mutations and derivatives thereof. The *TMPRSS2* promoter does not have to be the full-length wild type promoter. One skilled in the art knows how to derive fragments from a *TMPRSS2* promoter and test them for the desired selectivity. A *TMPRSS2* promoter fragment of the present invention has promoter activity selective for prostate tumor cells, *i.e.* drives prostate tumor selective expression of an operatively linked coding sequence. The term "prostate tumor selective promoter activity" as used herein means that the promoter activity of a *TMPRSS2* promoter fragment of the present invention in prostate tumor cells is higher than in non-tumor cell types. Preferably, the *TMPRSS2* promoter of the invention is a mammalian *TMPRSS2* promoter and more preferably is a human *TMPRSS2* promoter (h*TMPRSS2*).

In one embodiment of the invention, the *TMPRSS2* promoter consists essentially of the following sequence, which is a 1992 bp fragment of the h*TMPRSS2* promoter:

GGTCTTTGAGGGTTTCCCACTGGGGCAGAGCGCAGGGCAGTGTGGGGGATGGGGAGGGCT
GGGGCATATTGGGGACAATCCCTGTTGTGGGTGCCACCAGCAGACCACAAAAGGTGCCAA
AGGAGGGAGTGAGAGGGAAGGGTTTACTAGGAAAGTCCTGCCCCACAGGAGGCTTCCGGG
AGAGCTTCCCAGGAAGGGAGCAGTGAGCCAAGGCAGCCTGGGATGGGACTGAATGGGGCT
TTTTTCTGCTTCCACCTCATTTTAAAGCAAATCATGTTGATTTGTATATTATGCAGGGGA
GGAGAAAACATGCTTCCCCTCTACCCTTCTAGTTCGTTCAGCTAGTCTACAAATTAAATT
GGCATCAAATAGATGAACAGGAGAAAAGCTGTTTTAATGTATGTACTCACAGATGGGAAT
CCCACAAGAATATGAGACTTAAAGAACAGGCCAGGTGAGTGAGGGGTCCAGTGCGGGGGC
TCACACCTGTAATCCCAGCACTTTGGGAGGCCAAGGCAGGTGGATCGCTTGAGCCCAGAT
TGGAGACCAGTCTGGGTAACATAGGGAGACCCCCATCTCTACAAAAAATTAAATTAGCTG
GGCGTGGTGGCACACGCCTCTAGTCCCAGCTACTCGGGAGGCTGAGGTGGGAGGATTACT
TGTGCCCTGGGAGGTTGAAGCTGCCATGAGCCATGATTGAGCCACTGCACTCCAACCTGG
GCAACAGAGTGAGACCCCTTCTGGGGGAAAAAAAAACACACGAAAAAAAAAAGGTGCAGC
AGCCCGATGATTGAGGCTTATCTGTCACTCTGAGTGACAGAAAGAAATGGGGGTTTGAGG
CTTCTGGGGAGCGGTGGAGGAGTGAGGGGAGCGTGAGGAGAGGAGGTGTCTGGTGAACGC
AGGTTGCCGTGTGAGGCAGATAAAAGTTTCCCAGGTGATAAAAGTTGTCCGGGAACAGCT
CTCTTCCTGGTACAGATCTGCTGACTAACAAACATTTCCTTTATAGGTGCAAATTTCCTT
TACAAAAGGGCATTTTCTCAGAGGTACTCTGGTGTCTGCAGTTCCTCAACATAACCAGTT
CCAAATCATCAATGTGCCAAAGAGGACTATGTTGGGGTAGCAGATTCTGGTCTCCTCCAG
TCCTACTTGGGGTGATGAATTCTGGTCTACGGTCCTATTAAATTCTGGTGAATTCTGAGT
CCCCACAATTGCAAACATTAGAAAGAACCTCTCAAGTGCCCGGGAACAGCCACGTCTTCC
TGGCTGAGGTGTGTCCCACCACTTCCTCACTCCCGCCCTGGCCGGTGGTGCCGAGAGACC
TGGGACCATCCGGGGGAGCCCTTTCCACCGGACGCTGGTGGGGGCCAAGAAATGCCAGCC
TAGGCGGACTGGGGAGGGTCTTGGGCGTCCGGCGCTGTGTCCCCGCCACTCGTGCTTGGG
CCAGCAGTCCCCAAGGCCTACTCCTGGGTCCTTGCCCAGAGGCTACAGTGGGTTCCCCGG
AGGCCAAGACGGGGCCGGCCGCCTACAGGAGCTCGTGAGGTAGCAGCTCCGGGGGCTCAC
CCAGGACTCCAGGAGCGCTCCCCAGAATCCCCTTCCTTAACCCAAACTCGAGCCCTCGGG
CAGCGCTGCGCCGCGGACCGGAGAGGGGCAGGTTGGCCGCTGTGGCCGGGCCCGGGAAGC
GCCCCAGAGTCCCTTATGGGTCCCTCCGCAGCCGGGGTTGAGCCAGGCAGGGAACCCGTC
CCGGACTTCCCTTGGGAAACGCCTCCTCCCGCCGCCCCCGCCCCCGCCCGCCCAGGGTGA
CCCGCGACCCGCTTGGGGGTGTCGCCCTGGACCCTGGGACACCGCCTCCTGAGATTAAAG
CGAGAGCCAGGGCGGGCCGGGCCGAGTAGGCGCGAGCTAAGCAGGAGGCGGAGGCGGAGG
CGGAGGGCGAGGGGCGGGGAGCGCCGCCTGGAGCGCGGCAGGTGAGCGGCGCCGGTACCA
GGGTCCCGGCTC (SEQ ID NO:1)

In other embodiments, a *TMPRSS2* promoter according to the present invention has at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or 100% identity to the sequence shown in SEQ ID NO:1, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. Preferably, the given % sequence identity exists over a region of the sequences that is at least about 50 nucleotides in length, more preferably over a region of at least about 100 nucleotides, and even more preferably over a region of at least about 200 nucleotides. Most preferably, the given % sequence identity exists over the entire length of the sequences.

For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48: 443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), by the BLAST algorithm, Altschul *et al., J. Mol. Biol.* 215: 403-410 (1990), with software that is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/), or by visual inspection (see generally, Ausubel *et al., infra*). For purposes of the present invention, optimal alignment of sequences for comparison is most preferably conducted by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2: 482 (1981).

In another embodiment, a *TMPRSS2* promoter according to the present invention has a full-length complement that hybridizes to the sequence shown in SEQ ID NO:1 under stringent conditions. The phrase "hybridizing to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (*e.g.*, total cellular) DNA or RNA. "Bind(s) substantially" refers to complementary hybridization between a probe nucleic acid and a target nucleic acid and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target nucleic acid sequence.

"Stringent hybridization conditions" and "stringent wash conditions" in the context of nucleic acid hybridization experiments such as Southern and Northern hybridizations are sequence dependent, and are different under different environmental parameters. Longer sequences hybridize at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes part 1 chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays" Elsevier, New York. Generally,

highly stringent hybridization and wash conditions are selected to be about 5°C to 20°C (preferably 5°C) lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. Typically, under highly stringent conditions a probe will hybridize to its target subsequence, but to no other sequences.

The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of stringent hybridization conditions for hybridization of complementary nucleic acids that have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formamide with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.1 5M NaCl at 72°C for about 15 minutes. An example of stringent wash conditions is a 0.2xSSC wash at 65°C for 15 minutes (see, Sambrook, infra, for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 1xSSC at 45°C for 15 minutes. An example low stringency wash for a duplex of, *e.g.*, more than 100 nucleotides, is 4-6xSSC at 40°C for 15 minutes. For short probes (*e.g.*, about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less than about 1.0M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3, and the temperature is typically at least about 30°C. Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. In general, a signal to noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization.

The term "*TERT* promoter" refers to the native *TERT* promoter and functional fragments, mutations and derivatives thereof. The *TERT* promoter does not have to be the full-length wild type promoter. One skilled in the art knows how to derive fragments from a *TERT* promoter and test them for the desired selectivity. Preferably, the *TERT* promoter of the invention is a mammalian *TERT* promoter and more preferably is a human *TERT* promoter (*hTERT*). See WO 98/14593 and WO 00/46355, both of which are incorporated herein by reference.

In one embodiment of the invention, the *TERT* promoter consists essentially of the following 397 bp fragment of the *hTERT* promoter:

ccctcgctggcgtccctgcaccctgggagcgcgagcggcgcgcgggcggggaagcgcggcccag
acccccgggtccgcccggagcagctgcgctgtcggggccaggccgggctcccagtggattcgcg
ggcacagacgcccaggaccgcgcttcccacgtggcggagggactggggacccgggcacccgtcc
tgccccttcaccttccagctccgcctcctccgcgcggaccccgccccgtcccgacccctcccgg
gtccccggcccagccccctccgggccctcccagcccctcccccttcctttccgcggccccgccct
ctcctcgcggcgcgagtttcaggcagcgctgcgtcctgctgcgcacgtgggaagccctggcccc
ggccacccccgcg (SEQ ID NO:2)

In another embodiment of the invention, the *TERT* promoter consists essentially of the following 245 bp fragment of the *hTERT* promoter:

ccccacgtggcggagggactggggacccgggcacccgtcctgcccccttcaccttccagctccgc
ctcctccgcgcggaccccgccccgtcccgacccctcccgggtccccggcccagccccctccgggg
ccctcccagccccctcccccttcctttccgcggccccgccctctcctcgcggcgcgagtttcaggc
agcgctgcgtcctgctgcgcacgtgggaagccctggccccggccacccccgcg (SEQ ID NO:3)

The present invention provides novel adenoviral vectors based on the oncolytic adenoviral vector strategy as described in WO 96/17053 and WO 99/25860, the disclosures of which are hereby incorporated by reference in their entireties. In particular, oncolytic adenoviral vectors are disclosed in which expression of an adenoviral gene, which is essential for replication, is controlled by a regulatory region that is selectively transactivated in cancer cells. In accordance with the present invention, such a cancer selective regulatory regulatory region is a *TMPRSS2* promoter described in further detail herein. The invention further comprises adenoviral vector particles, which comprise the viral vectors of the invention.

The adenoviral particles of the invention are made by standard techniques known to those skilled in the art. Adenoviral vectors are transferred into packaging cells by techniques known to those skilled in the art. Packaging cells typically complement any functions deleted from the wildtype adenoviral genome. The production of such particles requires that the vector be replicated and that those proteins necessary for assembling an infectious virus be produced. The packaging cells are cultured under conditions that permit the production of the desired viral vector particle. The particles are recovered by standard techniques. The preferred packaging cells are those that have been designed to limit homologous recombination that could lead to wildtype adenoviral particles. Cells that may be used to produce the adenoviral particles of the invention include the human embryonic kidney cell line 293 (Graham *et al., J. Gen. Virol.* 36:59-72 (1977)), the human embryonic retinoblast cell line PER.C6 (U.S. Patent Nos. 5,994,128 and 6,033,908; Fallaux *et al., Hum. Gene Ther.* 9: 1909-1917 (1998)), and the human cervical tumor-derived cell line HeLa-S3 (co-pending provisional patent application of Kadan *et*

al., docket number 4-33160P1, filed on the same day as this application, entitled "Method For Producing Oncolytic Adenoviruses").

The present invention contemplates the use of all adenoviral serotypes to construct the oncolytic vectors and virus particles according to the present invention. In a preferred embodiment, the adenoviral nucleic acid backbone is derived from adenovirus serotype 2(Ad2), 5 (Ad5) or 35 (Ad35), although other serotype adenoviral vectors can be employed. Adenoviral stocks that can be employed according to the invention include any adenovirus serotype. Adenovirus serotypes 1 through 47 are currently available from American Type Culture Collection (ATCC, Manassas, VA), and the invention includes any other serotype of adenovirus available from any source including those serotypes listed in Table 1. The adenoviruses that can be employed according to the invention may be of human or non-human origin. For instance, an adenovirus can be of subgroup A (*e.g.*, serotypes 12, 18, 31), subgroup B (*e.g.*, serotypes 3, 7, 11, 14, 16, 21, 34, 35), subgroup C (*e.g.*, serotypes 1, 2, 5, 6), subgroup D (*e.g.*, serotypes 8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-47), subgroup E (serotype 4), subgroup F (serotype 40, 41), or any other adenoviral serotype.

TABLE 1

Examples Of Human And Animal Adenoviruses Including The American Type Culture Collection Catalog # For A Representative Virus Of The Respective Classification

Adenovirus Type 21 ATCC VR-1099	SA7 (Simian adenovirus 16) ATCC VR-941
SA18 (Simian adenovirus 18) ATCC VR-943	Frog adenovirus (FAV-1) ATCC VR-896
SA17 (Simian adenovirus 17) ATCC VR-942	Adenovirus type 48 (candidate) ATCC VR-1406
Adenovirus Type 47 ATCC VR-1309	Adenovirus Type 42 ATCC VR-1304
Adenovirus Type 44 ATCC VR-1306	Adenovirus Type 49 (candidate) ATCC VR-1407
Avian adenovirus Type 4 ATCC VR-829	Adenovirus Type 43 ATCC VR-1305
Avian adenovirus Type 5 ATCC VR-830	Avian adenovirus Type 6 ATCC VR-831
Avian adenovirus Type 7 ATCC VR-832	Avian adenovirus Type 3
Avian adenovirus Type 8 ATCC VR-833	Bovine adenovirus Type 3 ATCC VR-639
Avian adenovirus Type 9 ATCC VR-834	Bovine adenovirus Type 6 ATCC VR-642
Avian adenovirus Type 10 ATCC VR-835	Canine adenovirus ATCC VR-800
Avian adenovirus Type 2 ATCC VR-827	Bovine adenovirus Type 5 ATCC VR-641
Adenovirus Type 45 ATCC VR-1307	Adenovirus Type 36 ATCC VR-913
Adenovirus Type 38 ATCC VR-988	Ovine adenovirus type 5 ATCC VR-1343
Adenovirus Type 46 ATCC VR-1308	Adenovirus Type 29 ATCC VR-272
Simian adenovirus ATCC VR-541	Swine adenovirus ATCC VR-359

Bovine adenovirus Type 4 ATCC VR-640
Bovine adenovirus Type 8 ATCC VR-769
Bovine adenovirus Type 7 ATCC VR-768
Adeno-associated virus Type2 (AAV-2H) ATCC VR-680
Adenovirus Type 4 ATCC VR-4
Adeno-associated virus Type3 (AAV-3H) ATCC VR-681
Peromyscus adenovirus ATCC VR-528
Adenovirus Type 15 ATCC VR-661
Adenovirus Type 20 ATCC VR-662
Chimpanzee adenovirus ATCC VR-593
Adenovirus Type 31 ATCC VR-357
Adenovirus Type 25 ATCC VR-223
Chimpanzee adenovirus ATCC VR-592
Chimpanzee adenovirus ATCC VR-591
Adenovirus Type 26 ATCC VR-224
Adenovirus Type 19 ATCC VR-254
Adenovirus Type 23 ATCC VR-258
Adenovirus Type 28 ATCC VR-226
Adenovirus Type 6 ATCC VR-6
Adenovirus Type 2 Antiserum: ATCC VR-1079
Adenovirus Type 6 ATCC VR-1083
Ovine adenovirus Type 6 ATCC VR-1340
Adenovirus Type 3 ATCC VR-847
Adenovirus Type 7 ATCC VR-7
Adenovirus Type 39 ATCC VR-932
Adenovirus Type 3 ATCC VR-3
Bovine adenovirus Type 1 ATCC VR-313
Adenovirus Type 14 ATCC VR-15
Adenovirus Type 1 ATCC VR-1078
Adenovirus Type 21 ATCC VR-256
Adenovirus Type 18 ATCC VR-1095
Baboon adenovirus ATCC VR-275
Adenovirus Type 10 ATCC VR-11
Adenovirus Type 33 ATCC VR-626
Adenovirus Type 34 ATCC VR-716
Adenovirus Type 15 ATCC VR-16
Adenovirus Type 22 ATCC VR-257
Adenovirus Type 24 ATCC VR-259
Adenovirus Type 17 ATCC VR-1094
Adenovirus Type 4 ATCC VR-1081
Adenovirus Type 16 ATCC VR-17
Adenovirus Type 17 ATCC VR-18
Adenovirus Type 16 ATCC VR-1093
Bovine adenovirus Type 2 ATCC VR-314
SV-30 ATCC VR-203
Adenovirus Type 32 ATCC VR-625
Adenovirus Type 20 ATCC VR-255
Adenovirus Type 13 ATCC VR-14
Adenovirus Type 14 ATCC VR-1091
Adenovirus Type 18 ATCC VR-19
SV-39 ATCC VR-353
Adenovirus Type 11 ATCC VR-849
Duck adenovirus (Egg drop syndrome) ATCC VR-921
Adenovirus Type 1 ATCC VR-1
Chimpanzee adenovirus ATCC VR-594
Adenovirus Type 15 ATCC VR-1092
Adenovirus Type 13 ATCC VR-1090
Adenovirus Type 8 ATCC VR-1368
SV-31 ATCC VR-204
Adenovirus Type 9 ATCC VR-1086
Mouse adenovirus ATCC VR-550
Adenovirus Type 9 ATCC VR-10
Adenovirus Type 41 ATCC VR-930
C1 ATCC VR-20
Adenovirus Type 40 ATCC VR-931
Adenovirus Type 37 ATCC VR-929
Marble spleen disease virus
Adenovirus Type 35 ATCC VR-718
SV-32 (M3) ATCC VR-205
Adenovirus Type 28 ATCC VR-1106
Adenovirus Type 10 ATCC VR-1087
Adenovirus Type 20 ATCC VR-1097
Adenovirus Type 21 ATCC VR-1098
Adenovirus Type 25 ATCC VR-1103
Adenovirus Type 26 ATCC VR-1104
Adenovirus Type 31 ATCC VR-1109

Adenovirus Type 19 ATCC VR-1096
SV-36 ATCC VR-208
SV-38 ATCC VR-355
SV-25 (M8) ATCC VR-201
SV-15 (M4) ATCC VR-197
Adenovirus Type 22 ATCC VR-1100
SV-23 (M2) ATCC VR-200
Adenovirus Type 11 ATCC VR-12
Adenovirus Type 24 ATCC VR-1102
Avian adenovirus Type 1
SV-11 (M5) ATCC VR-196
Adenovirus Type 5 ATCC VR-5
Adenovirus Type 23 ATCC VR-1101
SV-27 (M9) ATCC VR-202
Avian adenovirus Type 2 (GAL) ATCC VR-280
SV-1 (M1) ATCC VR-195
SV-17 (M6) ATCC VR-198
Adenovirus Type 29 ATCC VR-1107
Adenovirus Type 2 ATCC VR-846
SV-34 ATCC VR-207
SV-20 (M7) ATCC VR-199
SV-37 ATCC VR-209
SV-33 (M10) ATCC VR-206
Avian adeno-associated virus ATCC VR-865
Adeno-associated (satellite) virus Type 4 ATCC VR-646
Adenovirus Type 30 ATCC VR-273
Adeno-associated (satellite) virus Typel ATCCVR-645
Infectious canine hepatitis (Rubarth's disease)
Adenovirus Type 27 ATCC VR-1105
Adenovirus Type 12 ATCC VR-863
Adeno-associated virus Type 2
Adenovirus Type 7a ATCC VR-848

In one aspect the present invention provides a recombinant adenoviral vector comprising an adenoviral nucleic acid backbone, wherein said nucleic acid backbone comprises in sequential order: a left ITR, a termination signal sequence, a cancer selective regulatory region of the invention that is operatively linked to a first gene essential for replication of the recombinant adenoviral vector, an adenoviral packaging signal, and a right ITR.

In another aspect the present invention provides a recombinant adenoviral vector comprising an adenoviral nucleic acid backbone, wherein said nucleic acid backbone comprises in sequential order: a left ITR, an adenoviral packaging signal, a termination signal sequence, a cancer selective regulatory region of the invention that is operatively linked to a first gene essential for replication of the recombinant adenoviral vector, and a right ITR.

In another aspect, the present invention provides a recombinant adenoviral vector comprising an adenoviral nucleic acid backbone, wherein said nucleic acid backbone comprises in sequential order: a left ITR, a termination signal sequence, a first cancer selective regulatory region operatively linked to a first gene essential for replication of the recombinant adenoviral vector, a second cancer selective regulatory region operatively linked to a second gene essential for replication, an adenoviral packaging signal, and a right ITR.

In another aspect, the present invention provides a recombinant adenoviral vector comprising an adenoviral nucleic acid backbone, wherein said nucleic acid backbone comprises in sequential order: a left ITR, an adenoviral packaging signal, a termination signal sequence, a

first cancer selective regulatory region operatively linked to a first gene essential for replication of the recombinant adenoviral vector, a second cancer selective regulatory region operatively linked to a second gene essential for replication, and a right ITR.

The first and second cancer selective regulatory regions may be essentially the same, derived from the same promoter(s) or may be derived from different promoters. In one embodiment, the first cancer selective regulatory region is a *TMPRSS2* promoter operatively linked to E1 and the second cancer selective regulatory region is an *hTERT* promoter or an E2F-1 promoter operatively linked to E4. In another embodiment, the first cancer selective regulatory region is an *hTERT* promoter or an E2F-1 promoter operatively linked to E1 and the second cancer selective regulatory region is a *TMPRSS2* promoter operatively linked to E4.

One exemplary vector comprises an Ad5 nucleic acid backbone, wherein the backbone comprises in sequential order a left ITR, an adenoviral packaging signal, an SV40 early polyA site, a *TMPRSS2* promoter operatively linked to the E1a gene, a telomerase promoter operatively linked to the E4 gene, and a right ITR.

The recombinant adenoviral vectors of this invention are useful as therapeutics for cancer therapy. As demonstrated herein, *TMPRSS2* is overexpressed in prostate tumor cells. Thus, the vectors of the invention preferentially kill prostate tumor cells. Furthermore, such vectors exhibit a favorable toxicity profile, which is clinically acceptable for the condition to be treated. Without wishing to be limited by theoretical considerations, the selective regulation of viral replication by a *TMPRSS2* promoter, which may be shielded from readthrough transcription by an upstream termination signal sequence, avoids toxicity that would occur if it replicated in non-target tissues, allowing for the favorable efficacy/toxicity profile.

The selectivity of the regulation of viral replication by a *TMPRSS2* promoter may be further enhanced in vectors of the invention via positioning of the packaging signal downstream of the *TMPRSS2* promoter-linked gene essential for replication. This positioning provides for the possibility to delete sequences of the adenoviral backbone that are located upstream of the *TMPRSS2* promoter-linked gene and that would encompass the packaging signal in its wildtype position. Such deletions can improve the selectivity of regulation of viral replication by a *TMPRSS2* promoter. See WO 02/067861 and WO 02/068627, both of which are incorporated herein by reference.

In a further embodiment, the oncolytic adenoviral vectors of the invention are designed to also be dependent on the over expression of telomerase in many cancers (~85% of all cancers). Like the intratumoral oncolytic adenovirus described above, the adenovirus of this

embodiment utilizes a *TMPRSS2* promoter to control expression of the adenoviral E1a gene and an *hTERT* (human telomerase reverse transcriptase) promoter to control expression of the adenoviral E4 gene, thereby increasing tumor selectivity.

In one embodiment, the recombinant viral vector of the invention comprises a termination signal sequence. The termination signal sequence increases the therapeutic effect because it reduces replication and toxicity of the oncolytic adenoviral vectors in non-target cells. Oncolytic vectors of the present invention with a polyadenylation signal inserted upstream of the E1a coding region have been shown to be superior to their non-modified counterparts as they have demonstrated the lowest level of E1a expression in nontarget cells. Thus, insertion of a polyadenylation signal sequence to stop nonspecific transcription from the left ITR improves the specificity of E1a expression from the respective promoter. Insertion of the polyadenylation signal sequences reduces replication of the oncolytic adenoviral vector in nontarget cells and therefore toxicity. A termination signal sequence may also be placed before (5') any promoter in the vector. In one embodiment, the terminal signal sequence is placed before a heterologous promoter operatively linked to the E4 gene, *e.g.* an *hTERT* promoter.

In another embodiment, the recombinant viral vector further comprises a deletion upstream of the termination signal sequence, such as a deletion between nucleotides 103 and 551 of the adenoviral type 5 backbone or corresponding positions in other serotypes. A deletion in the packaging signal 5' to the termination signal sequence may be such that the packaging signal becomes non-functional. In one embodiment, the deletion comprises a deletion 5' to the termination signal sequence wherein the deletion spans at least the nucleotides 189 to 551. In another embodiment the deletion comprises a deletion 5' to the termination signal sequence wherein the deletion spans at least nucleotides 103 to 551 (Figure 2 of WO 02/067861 and WO 02/068627). In this particular embodiment, it is preferred that the packaging signal is located (*i.e.* re-inserted) at a position 3' to the termination signal sequence and downstream of the *TMPRSS2* promoter-linked gene essential for replication.

In one embodiment, the invention further comprises a mutation or deletion in the E3 region. However, in an alternative embodiment, all or a part of the E3 region may be preserved or re-inserted in the oncolytic adenoviral vector. See, e.g., U.S. Pat. No. 6,495,130, incorporated herein by reference. Presence of all or a part of the E3 region may decrease the immunogenicity of the adenoviral vector. It also may increase cytopathic effect in tumor cells and decrease toxicity to normal cells. Preferably, the vector expresses more than half of the E3 proteins.

In an alternative embodiment, the invention further comprises a mutation or deletion in the E1b gene. Preferably the mutation or deletion in the E1b gene is such that the E1b-19kD protein becomes non-functional. This modification of the E1b region may be combined with vectors where all or a part of the E3 region is present.

In another embodiment, the oncolytic adenoviral vector further comprises at least one heterologous coding sequence, such as a therapeutic gene coding sequence. The therapeutic gene, preferably in the form of cDNA, can be inserted in any position that does not adversely affect the infectivity or replication of the vector. Preferably, it is inserted in the E3 region in place of at least one of the polynucleotide sequences coding for the E3 proteins. For example, the therapeutic gene may be inserted in place of the 19kD or 14.7 kD E3 gene.

A therapeutic gene can be one that exerts its effect at the level of RNA or protein. Therapeutic genes that may be introduced into the adenovirus include a factor capable of initiating apoptosis, antisense or ribozymes, which among other capabilities may be directed to mRNAs encoding proteins essential for proliferation, such as structural proteins, transcription factors, polymerases, etc., genes encoding cytotoxic proteins, genes that encode an engineered cytoplasmic variant of a nuclease (*e.g.* RNase A) or protease (*e.g.* trypsin, papain, proteinase K, carboxypeptidase, etc.), or encode the Fas gene, and the like.

Other therapeutic genes of interest include, but are not limited to, immunostimulatory, anti-angiogenic, and suicide genes. Immunostimulatory genes include, but are not limited to, genes that encode cytokines (GM-CSF, IL1, IL2, IL4, IL5, IFNα, IFNγ, TNFα, IL12, IL18, and flt3), proteins that stimulate interactions with immune cells (B7, CD28, MHC class I, MHC class II, TAPs), tumor-associated antigens (immunogenic sequences from MART-1, gp100(pmel-17), tyrosinase, tyrosinase-related protein 1, tyrosinase-related protein 2, melanocyte-stimulating hormone receptor, MAGE1, MAGE2, MAGE3, MAGE12, BAGE, GAGE, NY-ESO-1, β-catenin, MUM-1, CDK-4, caspase 8, KIA 0205, HLA-A2R1701, α-fetoprotein, telomerase catalytic protein, G-250, MUC-1, carcinoembryonic protein, p53, Her2/neu, triosephosphate isomerase, CDC-27, LDLR-FUT, telomerase reverse transcriptase, and PSMA), cDNAs of antibodies that block inhibitory signals (CTLA4 blockade), chemokines (MIP1α, MIP3α, CCR7 ligand, and calreticulin), and other proteins. Anti-angiogenic genes include, but are not limited to, genes that encode METH-1, METH -2, TrpRS fragments, proliferin-related protein, prolactin fragment, PEDF, vasostatin, various fragments of extracellular matrix proteins and growth factor/cytokine inhibitors. Various fragments of extracellular matrix proteins include, but are not limited to, angiostatin, endostatin, kininostatin,

fibrinogen-E fragment, thrombospondin, tumstatin, canstatin, and restin. Growth factor/cytokine inhibitors include, but are not limited to, VEGF/VEGFR antagonist, sFlt-1, sFlk, sNRP1, angiopoietin/tie antagonist, sTie-2, chemokines (IP-10, PF-4, Gro-beta, IFN-gamma (Mig), IFNα, FGF/FGFR antagonist (sFGFR), Ephrin/Eph antagonist (sEphB4 and sephrinB2), PDGF, TGFβ and IGF-1.

A "suicide gene" encodes a protein that itself can lead to cell death, as with expression of diphtheria toxin A, or the expression of the protein can render cells selectively sensitive to certain drugs, *e.g.*, expression of the Herpes simplex thymidine kinase gene (HSV-TK) renders cells sensitive to antiviral compounds, such as acyclovir, gancyclovir and FIAU (1-(2-deoxy-2-fluoro-β-D-arabinofuranosil)-5-iodouracil). Other suicide genes include, but are not limited to, genes that encode carboxypeptidase G2 (CPG2), carboxylesterase (CA), cytosine deaminase (CD), cytochrome P450 (cyt-450), deoxycytidine kinase (dCK), nitroreductase (NR), purine nucleoside phosphorylase (PNP), thymidine phosphorylase (TP), varicella zoster virus thymidine kinase (VZV-TK), and xanthine-guanine phosphoribosyl transferase (XGPRT). Alternatively, the therapeutic gene can exert its effect at the level of RNA, for instance, by encoding an antisense message or ribozyme, a protein that affects splicing or 3' processing (*e.g.*, polyadenylation), or a protein that affects the level of expression of another gene within the cell, *e.g.* by mediating an altered rate of mRNA accumulation, an alteration of mRNA transport, and/or a change in post-transcriptional regulation. The addition of a therapeutic gene to the virus results in a virus with an additional antitumor mechanism of action. Thus, a single entity (*i.e.*, the virus carrying a therapeutic transgene) is capable of inducing multiple antitumor mechanisms.

Alternately, the therapeutic gene coding sequence encodes thymidine kinase, Nos, FasL, sFasR (soluble Fas receptor), or granulocyte macrophage colony stimulating factor (GM-CSF; U.S. Patent No. 5,908,763, incorporated herein by reference).

The therapeutic gene coding sequence is under the control of a suitable promoter. Suitable promoters that may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter and/or the E3 promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the Rous Sarcoma Virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; and a tissue-selective promoter such as those disclosed in PCT/EP98/07380 (WO 99/25860). A *TMPRSS2* promoter may also be used.

The invention further comprises combinations of two or more transgenes with synergistic, complementary and/or nonoverlapping toxicities and methods of action. The resulting oncolytic adenovirus would retain the viral oncolytic functions and would, for example, additionally have the ability to induce immune and anti-angiogenic responses, etc.

In another embodiment, the adenoviral particles of the invention further comprise a targeting ligand included in a capsid protein of the particle. In one embodiment, the capsid protein is a fiber protein and the ligand is in the HI loop of the fiber protein. The adenoviral vector particle may also include other mutations to the fiber protein. Examples of these mutations include, but are not limited to those described in US application no. 10/351,890, WO 98/07877, WO 01/92299, and US Patent Nos. 5,962,311, 6,153,435, and 6,455,314. These include, but are not limited to mutations that decrease binding of the viral vector particle to a particular cell type or more than one cell type, enhance the binding of the viral vector particle to a particular cell type or more than one cell type and/or reduce the immune response to the adenoviral vector particle in an animal. In addition, the adenoviral vector particles of the present invention may also contain mutations to other viral capsid proteins. Examples of these mutations include, but are not limited to those described in US Patent Nos. 5,731,190, 6,127,525, and 5,922,315. Other mutated adenoviruses are described in U.S. Patent Nos. 6,057,155, 5,543,328 and 5,756,086.

Accordingly, in another aspect there is provided a method of selectively killing a neoplastic cell in a cell population that comprises contacting an effective amount of the viral vectors and/or viral particles of the invention with said cell population under conditions where the viral vectors and/or particles can transduce the neoplastic cells in the cell population, replicate, and kill the neoplastic cells.

The viral vectors of the invention are useful in studying methods of killing neoplastic cells *in vitro* or in animal models. Preferably, the cells are mammalian cells. More preferably, the mammalian cells are primate cells. Most preferably, the primate cells are human cells. In one embodiment of the invention, the recombinant viral vectors and/or particles selectively replicate in and lyse prostate cancer cells.

In a further aspect of the invention, a pharmaceutical composition comprising the recombinant viral vectors and/or particles of the invention and a pharmaceutically acceptable carrier is provided. Such compositions, which can comprise an effective amount of adenoviral vectors and/or particles of this invention in a pharmaceutically acceptable carrier, are suitable for local or systemic administration to individuals in unit dosage forms, sterile parenteral solutions

or suspensions, sterile non-parenteral solutions or oral solutions or suspensions, oil in water or water in oil emulsions and the like. Formulations for parenteral and non-parenteral drug delivery are known in the art. Compositions also include lyophilized and/or reconstituted forms of the adenoviral vectors and particles of the invention. Acceptable pharmaceutical carriers are, for example, saline solution, protamine sulfate (Elkins-Sinn, Inc., Cherry Hill, N.J.), water, aqueous buffers, such as phosphate buffers and Tris buffers, or Polybrene (Sigma Chemicel, St. Louis MO) and phosphate-buffered saline and sucrose. The selection of a suitable pharmaceutical carrier is deemed to be apparent to those skilled in the art from the teachings contained herein. These solutions are sterile and generally free of particulate matter other than the desired adenoviral virions. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. Excipients that enhance infection of cells by adenovirus may be included.

The viral vectors are administered to a host in an amount that is effective to inhibit, prevent, or destroy the growth of the tumor cells through replication of the viral vectors in the tumor cells. Such administration may be by systemic administration as hereinabove described, or by direct injection of the vectors in the tumor. In general, the vectors are administered systemically in an amount of at least 5×10^9 particles per kilogram body weight and in general, such an amount does not exceed 2.5×10^{12} particles per kilogram body weight. The vectors are administered intratumorally in an amount of at least 2×10^{10} particles and in general such an amount does not exceed 2×10^{13} particles. The exact dosage to be administered is dependent upon a variety of factors including the age, weight, and sex of the patient, and the size and severity of the tumor being treated. The viruses may be administered one or more times, times, which may be dependent upon the immune response potential of the host. Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. If necessary, the immune response may be diminished by employing a variety of immunosuppressants, so as to permit repetitive administration and/or enhance replication by reducing the immune response to the viruses. Antineoplastic adenoviral therapy of the present invention may be combined with other antineoplastic protocols.

Delivery can be achieved in a variety of ways, employing liposomes, direct injection, catheters, topical applications, inhalation, etc.

In yet another aspect, a method of treating a host organism having a neoplastic condition is provided, comprising administering a therapeutically effective amount of the composition of the invention to said host organism.

In a preferred embodiment of the invention, the neoplastic tissue is abnormally proliferating, and preferably malignant tumor tissue. Preferably, the viral vector is distributed essentially throughout the tissue or tumor mass due to its capacity for selective replication in the tumor tissue.

All neoplastic conditions are potentially amenable to treatment with the methods of the invention. Tumor types include, but are not limited to hematopoietic, pancreatic, neurologic, hepatic, gastrointestinal tract, endocrine, biliary tract, sinopulmonary, head and neck, soft tissue sarcoma and carcinoma, dermatologic, reproductive tract, respiratory, and the like. Preferred tumors for treatment are those with a high mitotic index relative to normal tissue. Preferred tumors are solid tumors. In one embodiment of the method of treatment, the neoplastic condition is prostate cancer.

In a preferred embodiment the host organism is a human patient. For human patients, if a therapeutic gene is included in the vector, the therapeutic gene may be of human origin although genes of closely related species that exhibit high homology and biologically identical or equivalent function in humans may be used if the gene does not produce an adverse immune reaction in the recipient. A therapeutic active amount of a nucleic acid sequence or a therapeutic gene is an amount effective at dosages and for a period of time necessary to achieve the desired result. This amount may vary according to various factors including but not limited to sex, age, weight of a subject, and the like.

The invention also provides for screening candidate drugs to identify agents useful for modulating the expression of *TMPRSS2* in cancer tissue, and hence useful for treating cancer. Appropriate host cells are those in which the regulatory region of *TMPRSS2* is capable of functioning. In one embodiment, the regulatory region of *TMPRSS2* is used to make a variety of expression vectors to express a marker that can then be used in screening assays. The expression vectors may be either self-replicating extrachromosomal vectors or vectors that integrate into a host genome. Generally, these expression vectors include a transcriptional and translational regulatory nucleic acid sequence of *TMPRSS2* operatively linked to a nucleic acid encoding a marker. The marker may be any protein that can be readily detected. It may be a detected on the basis of light emission, such as luciferase, color, such as β-galactosidase, enzyme activity, such

as alkaline phosphatase or antibody reaction, such as a protein for which an antibody exists. In addition, the marker system may be a viral vector or particle of the present invention.

In one embodiment, the viral vector or particle is used to assess the modulation of the *TMPRSS2* promoter. According to this embodiment, an effective amount of the viral vectors or viral particles of the invention is contacted with said cell population under conditions where the viral vectors or particles can transduce the neoplastic cells in the cell population, replicate, and kill the neoplastic cells. The candidate agent is either present in the culture medium for the test sample or absent for the control sample. The LD_{50} of the viral vectors or particles in the presence and absence of the candidate agent is compared to identify the candidate agents that modulate the expression of the *TMPRSS2* gene. If the level of expression is different as compared to similar viral vector controls lacking the *TMPRSS2* promoter, the candidate agent is capable of modulating the expression of *TMPRSS2* and is a candidate for treating cancers involving this gene and for further development of active agents on the basis of the candidate agent so identified.

In a second embodiment, the candidate agent is added to host cells containing the expression vector and the level of expression of the marker is compared with a control. If the level of expression is different, the candidate agent is capable of modulating the expression of *TMPRSS2* and is a candidate for treating cancers involving this gene and for further development of active agents on the basis of the candidate agent so identified.

Active agents so identified may also be used in combination treatments with oncolytic adenoviruses of the invention.

Having identified the *TMPRSS2* gene as being associated with cancer, a variety of assays may be executed. In an embodiment, assays may be run on an individual gene or protein level. That is, having identified a gene as up-regulated in cancer, candidate bioactive agents may be screened to modulate this gene's response; preferably to down-regulate the gene, although in some circumstances to up regulate the gene. "Modulation" thus includes both an increase and a decrease in gene expression. The preferred amount of modulation will depend on the original change of the gene expression in normal versus tumor tissue, with changes of at least 10%, preferably 50%, more preferably 100-300%, and in some embodiments 300-1000% or greater. Thus, if a gene exhibits a 4 fold increase in tumor compared to normal tissue, a decrease of about four fold is desired; a 10 fold decrease in tumor compared to normal tissue gives a 10 fold increase in expression for a candidate agent is desired.

The terms "candidate bioactive agent," "drug candidate" "compound" or grammatical equivalents as used herein describes any molecule, *e.g.*, protein, oligopeptide, small organic molecule, polysaccharide, polynucleotide, etc., to be tested for bioactive agents that are capable of directly or indirectly altering the cancer phenotype or the expression of a cancer sequence, including both nucleic acid sequences and protein sequences. In preferred embodiments, the bioactive agents modulate the expression profiles, or expression profile nucleic acids or proteins provided herein. In a particularly preferred embodiment, the candidate agent suppresses a cancer phenotype, for example to a normal prostate tissue fingerprint. Similarly, the candidate agent preferably suppresses a severe cancer phenotype. Generally a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, *i.e.*, at zero concentration or below the level of detection.

Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 100 and less than about 2,500 daltons. Preferred small molecules are less than 2000, or less than 1500 or less than 1000 or less than 500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Particularly preferred are peptides.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification to produce structural analogs.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics, immunology, cell biology, cell culture and transgenic biology, which are within the skill of the art. See, *e.g.*, Maniatis *et al.*, 1982, *Molecular Cloning* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York); Sambrook *et al.*, 1989, *Molecular Cloning*, 2nd Ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York); Sambrook and Russell, 2001, *Molecular Cloning*, 3rd Ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York); Ausubel *et al.*, 1992, *Current Protocols in Molecular Biology* (John Wiley & Sons, including periodic updates); Glover, 1985, *DNA Cloning* (IRL Press, Oxford); Anand, 1992, *Techniques for the Analysis of Complex Genomes*, Academic Press, New York; Guthrie and Fink, 1991, *Guide to Yeast Genetics and Molecular Biology* , Academic Press, New York; Harlow and Lane, 1988, *Antibodies*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York); Jakoby and Pastan, 1979; *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); *Transcription And Translation* (B. D. Hames & S. J. Higgins eds. 1984); *Culture Of Animal Cells* (R. I. Freshney, Alan R. Liss, Inc., 1987); *Immobilized Cells And Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide To Molecular Cloning* (1984); the treatise, *Methods In Enzymology* (Academic Press, Inc., N.Y.); *Gene Transfer Vectors For Mammalian Cells* (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); *Immunochemical Methods In Cell And Molecular Biology* (Mayer and Walker, eds., Academic Press, London, 1987); *Handbook Of Experimental Immunology*, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); Riott, *Essential Immunology*, 6th Edition, Blackwell Scientific Publications, Oxford, 1988; Hogan *et al.*, *Manipulating the Mouse Embryo*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

EXAMPLES

The present invention is described by reference to the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized.

EXAMPLE 1: Identification Of Cancer-Selective Genes By Gene Expression Profiling With Tumor / Normal Tissue Microarray Databases

A tumor/normal tissue microarray database including prostate tumors, normal prostates, colon tumors, normal colons, as well as normal lungs, normal livers, normal kidneys and one normal heart are analyzed. RNA samples are hybridized with more than 200,000 different oligonucleotide probes on Affymetrix human U95A v.2 chips. The transcript levels of more than 8000 known genes in the human genome are profiled for each sample. Differential expression of the genes (expressed as "average difference values") in tumor and normal tissues is determined using an algorithm that ranks the genes by criteria designed to identify genes that show low to high expression levels in a majority of samples from a cancer of interest, and that show an absence of expression in a majority of samples from non-target tissues. Non-target tissues include matched normal tissue for the particular cancer type (except prostate cancer), liver, lung, kidney and heart. These criteria are applied to the microarray data in Excel by setting the following parameters by which to rank the candidate gene average difference values: a mean level of expression in the tumor samples greater than 200 relative units, a mean level of expression in the normal matched tissue of less than 40 (except for prostate cancer), a mean level of expression in non-target tissues of less than 40, and having the above criteria met in >50% of the tumor and normal tissue samples.

The candidates are further evaluated for their expression levels in different cell lines to select cell lines that can be used as positive and negative controls for expression. The genes that fulfill the criteria for differential expression both in tumor tissues as well as in particular cell lines are determined to be candidate genes.

In an examplary study carried out according to the above, a tumor/normal tissue microarray database including 24 prostate tumors, 9 normal prostates, 21 colon tumors, 5 normal colons, as well as 5 normal lungs, 5 normal livers, 4 normal kidneys and one normal heart was

analyzed. The majority of the genes were expressed in at least one cell line representative of the original tumor, but not expressed in at least one other cell line. Characteristics of a prostate cancer-selective gene identified from the microarray data mining, *TMPRSS2*, are shown in Table 2 below. The promoter of *TMPRSS2* may be used to drive prostate cancer selective killing in the context of adenoviral vectors.

TABLE 2

Prostate Cancer Selective *TMPRSS2* Gene Identified By Expression Profiling

Gene Symbol	U95a chip probe set ID	Attributes	GeneBank acc.#	Unigen ID	mRNA
TMPRSS2	34996_at	Transmembrane protease, serine 2	AF329454	Hs.318545	NM_005656

EXAMPLE 2: Validation Of Selective Gene Expression In Tumor Target Versus Non-Target And Normal Cell Lines By Semi-Quantitative rt-PCR

Semi-quantitative rt-PCR is used to validate the differential expression of a candidate gene identified by microarray profiling. cDNA is prepared using RETROscript kit manufactured by Ambion Ltd (Austin, Texas) from each cell line. Primers used to amplify the *TMPRSS2* cDNA are: sense, 5'-CATTGCTACCTCAGTGCTCCTGGAAAC-3' (SEQ ID NO:4) and antisense, 5'-ACATCTTTCTCTTCTTCGCCGCCACC-3'; (SEQ ID NO:5). Multiplex PCR amplification is carried out in which the *TMPRSS2* cDNA is co-amplified with ribosomal 18s cDNA in the presence of *TMPRSS2*-specific primers and 18s-specific primers provided in QuantumRNA 18s Internal Standard kit manufactured by Ambion Ltd (Austin, Texas). The image intensity of the *TMPRSS2* transcript is normalized to the intensity of 18s transcript so that the level of *TMPRSS2* expression is semi-quantified and comparable among different cell lines. Sets of cell lines originally grouped into positive/negative cell lines by gene expression profiling are examined for their expression levels by the semi-quantitative rt-PCR method. The sensitivity by PCR amplification is higher than the one by microarray. This feature allows a high level of stringency in determining the negative cell lines. Thus, the PCR-identified differential pattern is used as a guideline for selecting positive/negative cell lines to be used as target/non-target cell lines to screen with the corresponding candidate oncolytic vectors for *in vitro* tumor-selective killing effects.

In an exemplary study carried out according to the above, the differential patterns detected by rt-PCR amplification were determined to be in reasonable agreement with the patterns identified by the expression profiling (see Table 3 below). In some cases, rt-PCR amplification detected a low level of expression that was not detected by the expression profiling. Nevertheless, the differential pattern maintained between the positive and negative cell lines. Three prostate tumor cell lines LNCap, C4-2 and Vcap were selected as *TMPRSS2*-positive cell lines, and SW620, H69, Hep3B and hPr-1 as the negative cell lines. Four normal human cell lines were all used for testing.

TABLE 3

TMPRSS2-Positive / Negative Cell Lines By rt-PCR vs. Microarray[1]

Cell line	Cell type	Expression by microarray	Expression by rt-PCR
LNCap	prostate tumor	+	+
C4-2	prostate tumor	+	+
Vcap	prostate bone metastasis tumor	+	+
SW620	colon carcinoma	-	±
H69	small cell lung cancer	-	-
Hep3B	hepatoma carcinoma	-	-
Wi38	human primary fibroblast	-	-
MRC5	human primary fibroblast	NA	-
HAEC	human aorta epithelial cell	NA	-
HRE	human renal epithelial cell	NA	-
hPr-1	transformed prostate cell	-	±
Prostate	prostate tissue	+	+

[1] Expression levels were scored as: + expressed, ± low expression, - not expressed. NA = data not available. Measurement in prostate tissue was included as a positive control of expression.

EXAMPLE 3: Promoter Annotation And Sequence Determination

Several web-based computational tools are applied to assist the annotation of a promoter in the human genome. An exon map of the gene in the GenBank database (available on the web at http://www.ncbi.nih.gov/cgi-bin) is used to determine the 5' end of an mRNA sequence. The first base pair of the exon 1 sequence usually indicates a transcription start site (TSS). The basal promoter region is generally defined as being within 500 bp upstream of the TSS. To include certain transcription factor binding sites further upstream of the basal promoter sequence, a

region containing 1.9 to 2 kb upstream and 100 to 250 bp downstream of the TSS is retrieved from the NCBI human genome database. *TMPRSS2* is located in the human genome at Chromosome 21, contig NT_011512. The sequence of the retrieved promoter region is shown as SEQ ID NO:1.

To predict the functionality of the retrieved promoter sequence, the sequence is analyzed for basal promoter elements and transcription factor binding sites using the computational tool linked to TRANSFAC, a transcription factor binding site database available on the web at http://www.genomatix.de/cgi-bin/eldorado/mail.pl. Examples of criteria that are used to determine which of the promoter fragments to test for tumor-selectivity are: 1) the sequence preferably contains either a TATA box or an Ebox/GC rich region in the proximal region, and 2) common transcription factor binding sites preferably occur as a cluster with each other forming a particular pattern. As an example of the prediction result, the promoter contains an Ebox/GC rich region within 600 bp upstream of TSS, while it does not contain a TATA box sequence. In addition, two common transcription factor binding sites, AP2F and EGRF, cluster within 250 bp upstream of TSS in this promoter. These characteristic components provide evidence for the sequence to be a basal promoter region.

As an example for the prediction of regulatory motif, several patterns common for prostate-selective promoter sequences are present in the *TMPRSS2* promoter region. These motifs include AP2F/SP1F, AP2F/ZBPF, EGRF/EKLF, EGRF/EGRF and SP1F/EGRF. These cluster patterns may belong to functional motifs for transcriptional activation of prostate-selective genes.

In addition, a group of binding sits for transcription factors Pax1, 5, & 6, E2TF, LTUP and NFkb are present in the *TMPRSS2* promoter/upstream sequence. These binding sites may be specific for the regulation of the *TMPRSS2* promoter in prostate tumor. These motif patterns are believed to be novel and are not known to have been been described in the literature.

EXAMPLE 4: Construction Of Oncolytic Adenoviral And Luciferase Reporter Vectors With A *TMPRSS2* Promoter Sequence

To evaluate the control of oncolytic vector replication by the *TMPRSS2* promoter, a 2 kb promoter sequence is isolated from human genomic DNA by PCR amplification and cloned into an adenoviral vector. The *TMPRSS2* promoter is cloned into an Ad5-based viral vector backbone in which the E1a promoter had been deleted and replaced with the SV40 early polyadenylation

signal. Primer sequences (underlined) for generating a *TMPRSS2* promoter are: sense primer with an NheI restriction site 5'-CATGTCATGCTAGCGGTCTTTGAGGGTTTCCCAC-3' (SEQ ID NO:6) and antisense primer with a PmeI restriction site 5'-AACTTTGTTTAAACGAGCCGGGACCCTGGTACCG-3' (SEQ ID NO:7). The *TMPRSS2* PCR fragment is digested with NheI and PmeI and ligated to NheI and PmeI sites between the SV40 polyadenylation signal and the adenoviral E1a coding region to generate a left end shuttle vector. This shuttle vector is then cut with FspI and SphI and the resulting fragment containing the *TMPRSS2* promoter and E1a is incorporated into an infectious Ad genomic plasmid through homologous recombination according to the method described in He *et al.*, *Proc Natl Acad Sci USA*, 1998, 95:2509-2514 to generate an *TMPRSS2* adenoviral vector.

To evaluate promoter selectivity at the level of transcriptional activation, the same 2 kb promoter sequence is also cloned into a modified luciferase expression cassette. This luciferase system features the luciferase coding sequence in the place of the E1 region in an adenoviral left shuttle plasmid. The promoter sequence is then cloned into restriction sites upstream of the luciferase coding sequence to drive luciferase expression. This modified reporter system more closely approximates the sequence context of the adenoviral ITR and packaging signal/enhancer that may influence the heterologous promoter activity. In addition, this system allows screening candidate promoters in a higher throughput scale, which accelerates the process of identifying selective motifs for further improvement of oncolytic vector selectivity and potency.

EXAMPLE 5: Tumor Killing Selectivity And Potency By MTS Assay With The *TMPRSS2* Adenoviral Vector

The *TMPRSS2* oncolytic adenoviral vector is evaluated by an MTS assay according to manufacturer's instructions (CellTiter 96® AQ_{ueous} Assay by Promega, Madison, WI) for its selectivity on target vs. non-target and normal cell lines. Wildtype Ad5 is included in the experiment as a normalization factor. Cells are seeded in 96-well dishes in 90µl volume one day prior to adenoviral infection. The next day, adenoviruses are diluted serially in the appropriate growth media and 10µl of each dilution is added to the wells. Cells are exposed to virus for seven to ten days, after which an MTS cytotoxicity assay (CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI)) is performed according to the manufacturer's instructions. Absorbance values are expressed as a percent of uninfected control and plotted versus vector dose. A sigmoidal dose-response curve is fit to the data and a lethal

dose-50 percent (LD_{50}) value is calculated for each replicate using GraphPad Prism software, version 3.0.

In an exemplary study carried out according to the above, the dose-response curves and LD_{50} data indicated that although the LD_{50} values of the *TMPRSS2* vector was higher than the values of the Ad5 vector for all of the cell lines tested, the values were closer to wildtype levels on tumor cell lines.

As shown in Table 4 below, the relative LD_{50} values of the *TMPRSS2* vector on prostate tumor cells were in the range of 0.2 to 0.7, indicating a vector killing potency close to that of the wildtype Ad5 vector. In contrast, the relative LD_{50} value was low on non-target tumor and normal cell lines, indicating low killing activity on these cells. Therefore, the *TMPRSS2* vector has both high tumor-killing selectivity and potency.

TABLE 4

Relative LD_{50} for *TMPRSS2* vector in various cell lines in an MTS assay.

Cell lines	Relative LD50 (Ad5 / *TMPRSS2*)
Target cells	
LNCap-1	0.204
LNCap-2	0.435
LNCap-3	0.677
C4-2-1	0.211
C4-2-2	0.167
C4-2-3	0.163
Vcap	0.187
Non-target cells (tumor)	
SW620	0.028
SW620, no CO2	0.059
H69	0.011
Non-target cells (normal)	
hPr-1	0.042
HRE	0.092
HAEC	0.057
MRC5	0.005
Wi38	0.011

In evaluating the selectivity of an oncolytic vector *in vitro*, comparison with a wildtype control such as Ad5 helps control for potential differences between cell lines such as transduction efficiency. Selectivity for tumor cell lines can be represented mathematically by a "selectivity index" value. In the current example, a selectivity index value for a vector is the

cytotoxicity of an oncolytic vector relative to Ad5 on tumor target versus non-target or normal cells. A selectivity index value above "1" is defined as having tumor cell selectivity. The higher the value, the better the selectivity. Tumor-killing selectivity is calculated based on the following equation:

$$\text{Selectivity Index} = \frac{LD_{50}\ Ad5_{\ target.tumor} \div LD_{50}\ OV_{\ target.tumor}}{LD_{50}\ Ad5_{\ nontarget.normal} \div LD_{50}\ OV_{\ nontarget.normal}}$$

In an exemplary study, the selectivity indices for the *TMPRSS2* vector was calculated on prostate tumor cell lines LNCap, C4-2 and Vcap vs. non-target tumor cell lines SW620, H69 and hPr-1 as well as the primary cell lines HRE, HAEC, MRC5 and Wi38. The experiments on the target prostate tumor cell lines were repeated three times in individual cell cultures and average selectivity index values were listed in Table 5. The high selectivity index value demonstrates that the *TMPRSS2* vector selectively kills prostate tumor cell lines LNCap, C4-2 and Vcap.

TABLE 5

Selectivity Index of *TMPRSS2* Vector

Target cell lines versus:	LNCap	C4-2	VCap
Non-target cell lines			
SW620-1	16.7	6.9	7.1
H69	40.2	16.5	17.2
HPr-1	10.3	4.3	4.4
Normal cell lines			
HRE	4.5	2.0	2.0
HAEC	7.7	3.2	3.3
MRC5	80.8	33.2	34.5
Wi38	40.0	24.6	17.1

In summary, a prostate-cancer selective *TMPRSS2* promoter sequence has been identified and the tumor-selective oncolytic effect on target tumor cell lines has been verified.

It will be appreciated that the methods and compositions of the instant invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent to the artisan that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid promoter comprising a nucleotide sequence selected from the group consisting of:

(a) the sequence shown in SEQ ID NO:1;

(b) a fragment of the sequence shown in SEQ ID NO:1, wherein said fragment has tumor selective promoter activity;

(c) a nucleotide sequence having at least 90% identity over its entire length to the sequence shown in SEQ ID NO:1, wherein said nucleotide sequence has tumor selective promoter activity; and

(d) a nucleotide sequence having a full-length complement that hybridizes under stringent conditions to the sequence shown in SEQ ID NO:1, wherein said nucleotide sequence has tumor selective promoter activity.

2. The isolated nucleic acid promoter according to claim 1, wherein said nucleic acid promoter comprises the sequence shown in SEQ ID NO:1.

3. The isolated nucleic acid promoter according to claim 1, wherein said nucleic acid promoter comprises a fragment of the sequence shown in SEQ ID NO:1, wherein said fragment has tumor selective promoter activity.

4. The isolated nucleic acid promoter according to claim 1, wherein said nucleic acid promoter comprises a nucleotide sequence having at least 90% identity over its entire length to the sequence shown in SEQ ID NO:1, wherein said nucleotide sequence has tumor selective promoter activity.

5. The isolated nucleic acid promoter according to claim 1, wherein said nucleic acid promoter comprises a nucleotide sequence having a full-length complement that hybridizes under stringent conditions to the sequence shown in SEQ ID NO:1, wherein said nucleotide sequence has tumor selective promoter activity.

6. The isolated nucleic acid promoter according to claim 1, which consists essentially of SEQ ID NO:1.

7. The isolated nucleic acid promoter according to any one of claims 1-6, wherein said promoter is prostate tumor selective.

8. A chimeric gene comprising the nucleic acid promoter according to any one of claims 1-7 operatively linked to the coding sequence of a gene of interest.

9. A vector comprising the chimeric gene according to claim 8.

10. The vector according to claim 9, wherein said vector is a viral vector.

11. The vector according to claim 9, wherein said vector is an adenoviral vector.

12. An isolated cell transduced with the vector according to claim 9.

13. A recombinant viral vector comprising an adenoviral nucleic acid backbone comprising: a left ITR, an adenoviral packaging signal, a *TMPRSS2* promoter operatively linked to the coding sequence of a gene essential for replication of the recombinant viral vector, and a right ITR.

14. The recombinant viral vector according to claim 13, wherein said *TMPRSS2* promoter is a human *TMPRSS2* promoter.

15. The recombinant viral vector according to claim 13, wherein said *TMPRSS2* promoter comprises a nucleotide sequence selected from the group consisting of:

(a) the sequence shown in SEQ ID NO:1;

(b) a fragment of the sequence shown in SEQ ID NO:1, wherein said fragment has prostate tumor selective promoter activity;

(c) a nucleotide sequence having at least 90% identity over its entire length to the sequence shown in SEQ ID NO:1, wherein said nucleotide sequence has prostate tumor selective promoter activity; and

(d) a nucleotide sequence having a full-length complement that hybridizes under stringent conditions to the sequence shown in SEQ ID NO:1, wherein said nucleotide sequence has prostate tumor selective promoter activity.

16. The recombinant viral vector according to claim 15, wherein said *TMPRSS2* promoter comprises the sequence shown in SEQ ID NO:1.

17. The recombinant viral vector according to claim 15, wherein said *TMPRSS2* promoter comprises a fragment of the sequence shown in SEQ ID NO:1, wherein said fragment has prostate tumor selective promoter activity.

18. The recombinant viral vector according to claim 15, wherein said *TMPRSS2* promoter comprises a nucleotide sequence having at least 90% identity over its entire length to the sequence shown in SEQ ID NO:1, wherein said nucleotide sequence has prostate tumor selective promoter activity.

19. The recombinant viral vector according to claim 15, wherein said *TMPRSS2* promoter comprises a nucleotide sequence having a full-length complement that hybridizes under stringent conditions to the sequence shown in SEQ ID NO:1, wherein said nucleotide sequence has prostate tumor selective promoter activity.

20. The recombinant viral vector according to claim 13, wherein said *TMPRSS2* promoter consists essentially of SEQ ID NO:1.

21. The recombinant viral vector according to claim 13, wherein the coding sequence of a gene essential for replication is selected from the group consisting of E1a, E1b, E2a, E2b and E4 coding sequences.

22. The recombinant viral vector according to claim 21, wherein the *TMPRSS2* promoter is operatively linked to one of either the E1a coding sequence or the E4 coding sequence.

23. The recombinant viral vector according to claim 22, further comprising a tissue-selective promoter operatively linked to the other of the E1a coding sequence or the E4 coding sequence.

24. The recombinant viral vector according to claim 23, wherein said tissue-selective promoter is an *hTERT* promoter, an E2F-1 promoter, or an osteocalcin promoter.

25. The recombinant viral vector according to claim 24, wherein said tissue-selective promoter is an *hTERT* promoter comprising SEQ ID NO:2 or SEQ ID NO:3.

26. The recombinant viral vector according to claim 13, wherein said adenoviral nucleic acid backbone is derived from adenovirus serotype 5 (Ad5) or serotype 35 (Ad35).

27. The recombinant viral vector according to claim 13, wherein said nucleic acid backbone further comprises a termination signal sequence upstream of the *TMPRSS2* promoter operatively linked to the coding sequence of a gene essential for replication of the recombinant viral vector.

28. The recombinant viral vector according to claim 27, wherein said termination signal sequence is the SV40 early polyadenylation signal sequence.

29. The recombinant viral vector according to claim 27, further comprising a deletion upstream of the termination signal sequence.

30. The recombinant viral vector according to claim 27, further comprising a deletion between nucleotides corresponding to nucleotides 103 and 551 of the adenoviral type 5 backbone.

31. The recombinant viral vector according to claim 13, wherein the E1b gene of said adenoviral nucleic acid backbone has been mutated or deleted.

32. The recombinant viral vector according to claim 31, wherein said mutation or deletion of the E1b gene results in the loss of the active 19kD protein expressed by the wild-type E1b gene.

33. The recombinant viral vector according to claim 13, wherein the E3 region of said adenoviral nucleic acid backbone has been mutated.

34. The recombinant viral vector according to claim 13, wherein the E3 region of said adenoviral nucleic acid backbone has been deleted.

35. The recombinant viral vector according to claim 13, further comprising a heterologous coding sequence.

36. The recombinant viral vector according to claim 35, wherein said heterologous coding sequence is in the E3 region of said adenoviral nucleic acid backbone.

37. The recombinant viral vector according to claim 36, wherein said heterologous coding sequence is inserted in place of the 19kD or 14.7 kD E3 gene.

38. The recombinant viral vector according to claim 35, wherein said heterologous coding sequence encodes an immunostimulatory protein.

39. The recombinant viral vector according to claim 38, wherein said immunostimulatory protein is a cytokine.

40. The recombinant viral vector according to claim 39, wherein said cytokine is GM-CSF.

41. The recombinant viral vector according to claim 35, wherein said heterologous coding sequence encodes an anti-angiogenic protein.

42. The recombinant viral vector according to claim 35, wherein said heterologous coding sequence is a suicide gene.

43. The recombinant viral vector according to claim 13, wherein said recombinant viral vector selectively replicates in tumor cells.

44. The recombinant viral vector according to claim 43, wherein said recombinant viral vector selectively replicates in prostate tumor cells.

45. The recombinant viral vector according to claim 43, wherein tumor-selectivity is at least about 3-fold as measured by E1a RNA levels in infected tumor vs. infected non-tumor cells.

46. A recombinant adenovirus particle comprising the recombinant viral vector according to any one of claims 13-45.

47. The recombinant adenovirus particle according to claim 46, wherein a capsid protein of said adenovirus particle comprises a targeting ligand.

48. The recombinant adenovirus particle according to claim 47, wherein said capsid protein is a fiber protein.

49. The recombinant adenovirus particle according to claim 48, wherein said targeting ligand is in the HI loop of said fiber protein.

50. A method of selectively killing a neoplastic cell, comprising contacting an effective number of recombinant adenovirus particles according to claim 46 with said cell under conditions where the recombinant adenovirus particles can transduce said cell.

51. The method of claim 50, wherein said neoplastic cell is a prostate tumor cell.

52. A pharmaceutical composition comprising the recombinant adenovirus particle according to claim 46 and a pharmaceutically acceptable carrier.

53. A method of treating a host organism having a neoplastic condition, comprising administering a therapeutically effective amount of the pharmaceutical composition according to claim 52 to said host organism.

54. The method of treatment according to claim 53, wherein the host organism is a human.

55. The method of treatment according to claim 53, wherein the neoplastic condition is prostate cancer.

56. The method of treatment according to claim 53, wherein administering a therapeutically effective amount of the pharmaceutical composition to said host organism comprises an intratumoral injection of a therapeutically effective dosage of the composition.

57. The method of treatment according to claim 53, wherein administering a therapeutically effective amount of the pharmaceutical composition to said host organism comprises systemic administration of a therapeutically effective dosage of the composition.

ABSTRACT

The present invention generally relates to substances and methods useful for the treatment of neoplastic disease. More specifically, it relates to cancer selective promoters and their use in oncolytic adenoviral vectors. The oncolytic adenoviral vectors are useful in methods of gene therapy. The promoters and the oncolytic adenoviral vectors are useful in methods for screening for compounds that modulate the expression of the cancer selective genes.

SEQUENCE LISTING

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